Arch. int. Pharmacodyn. **279**, 50–60 (1986)

The Effect of Repeated Administration of (−) Deprenyl on the Phenylethylamine-Induced Stereotypy in Rats

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Abstract—Phenylethylamine (PEA) in a dose of 40 mg/kg induces a moderate and short-lasting stereotype behaviour. The intensity and duration of PEA-induced stereotypy was strongly potentiated in rats with single selective doses of (−) deprenyl and J-508 (0.25 and 0.1 mg/kg s.c., respectively), which inhibit selectively the B form of MAO. Neither the increase of the single dose nor the daily administration of the selective doses of (−) deprenyl and J-508 for eight weeks caused a more intense or longer lasting potentiation of the PEA effect than single small dose of these drugs. Stopping administration of MAO-B inhibitors, normal sensitivity towards PEA returned after 3–4 weeks, in a parallel way with the recovery of MAO-B activity in the brain. Clorgyline, the selective inhibitor of MAO-A failed to influence the effect of PEA (0.25–2 mg/kg s.c.). The data support the view that the strong potentiation of the stimulatory effects of endogenous PEA via inhibition of its metabolism by (−) deprenyl may play a role in the antidepressant effect of this drug in man.

Introduction

Phenylethylamine (PEA) which was detected in trace amount in the brain of mammals (Inwang *et al.*, 1973; Borison *et al.*, 1975) is, as an indirectly acting sympathomimetic amine, a psychostimulant. Decreased PEA content has been found in the urine of depressed patients (Boulton and Milward, 1971; Fischer *et al.*, 1972; Mosnaim *et al.*, 1973). On the basis of these data, the role of PEA in affective behaviour has been suggested (Sabelli and Mosnaim, 1974), and it was claimed that PEA may

play the role of a physiological stimulant in the brain (Diamond and Borison, 1978; Sabelli *et al.*, 1978).

The discovery that clorgyline inhibits preferentially the oxidative deamination of serotonin (Johnston, 1968), while (−) deprenyl (Knoll *et al.*, 1965) inhibits selectively the deamination of benzylamine and m-iodobenzylamine (Knoll and Magyar, 1972) laid the foundation for our present knowledge of the two main forms of mitochondrial MAO. Using (−) deprenyl as a research tool it was demonstrated that PEA is selectively deaminated by the B-type MAO (Yang and Neff, 1973).

Mann *et al.* (1982) demonstrated that (−) deprenyl in the dose range which elevated dopamine and PEA concentration selectively in the brain, leaving noradrenaline and serotonin levels unchanged improved patients classified as having nonendogenous and endogenous depression. They emphasized that (−) deprenyl is a potentially important tool for identifying the biochemical subdivisions of depressive disorders.

The clinical observations clearly support the original view of Knoll (1976), who proposed the use of (−) deprenyl in combination with phenylalanine, the precursor of PEA, in the treatment of depression. According to a recent clinical study based on this suggestion, the simultaneous administration of (−) deprenyl and phenylalanine proved to be highly efficient in depressed patients (Birkmayer *et al.*, 1984). Thus it seems now to be of practical importance to learn more about the modification of PEA-induced central effects under the influence of (−) deprenyl. The detailed pharmacological and biochemical analyses with (−) deprenyl revealed the importance to administer this drug continously in small daily doses instead of the usually high single doses (Knoll, 1983).

With this in mind the present study was undertaken to investigate the effect of the daily administration of (−) deprenyl for up to eight weeks on the PEA-induced stereotypy, comparing it to the effect of another selective MAO-B inhibitor, J-508 (N-methyl-N-propargyl-(1-indonyl)-ammonium HCl) (Knoll *et al.*, 1978), and of a selective MAO-A inhibitor, clorgyline.

Methods

Animals and Treatment

Sprague-Dawley rats of both sexes, weighing 140–160 g at the beginning of the treatment and housed at constant temperature (20–21° C) under a standard 12 hr–12 hr light-dark cycle (light on at 6.00 a.m.) were used. Water and food (except for periods of testing) were available ad libitum.

In the first series of experiments graded doses of (−) deprenyl, J-508 and clorgyline were injected into groups of ten rats one hour prior to PEA. Control animals received physiological saline before PEA.

In the second series the animals received (−) deprenyl or clorgyline (0.25 mg/kg s.c.), respectively, or J-508 (0.1 mg/kg s.c.) daily for eight weeks. Control rats were treated similarly with an equivalent volume of physiological saline. The effect of PEA was checked at the end of the 1st, 2nd, 4th and 8th week of daily treatment, 24 hr after the last injection. Stopping the treatment with (−) deprenyl and J-508 at the end of the 8th week the PEA effect was further investigated 48 hr, 1, 2, 4 and 6 weeks later. Every stereotype experiment with PEA was carried out on 8–10 rats of each treated group, the interval between two PEA injections given to the same animal was at least one week. According to preliminary experiments the repeated administration of 40 mg/kg PEA s.c. weekly for five weeks failed to alter either the intensity or the duration of stereotypy.

Stereotype behaviour

A dose of PEA (40 mg/kg s.c.) was chosen, which elicited a stereotypy of moderate intensity. Stereotypy was assessed by the scoring system of Costall *et al.* (1972): 0 – no apparent drug effect, 1 – discontinuous sniffing, constant exploratory activity, 2 – continuous sniffing and periodic exploratory activity, 3 – continuous sniffing, discontinuous licking/or gnawing/or biting, very brief periods of locomotor activity, 4 – continuous licking/or gnawing/or biting, no exploratory activity.

For testing the stereotype behaviour the animals were placed individually in rectangular wire mash cages, separated from each other. Following a 30 min adaptation period they were injected with PEA and were observed every 10 min during a 60 sec interval till the cessation of stereotype behaviour. The maximal intensity of stereotypy shown by each animal was averaged and expressed as score max (arbitrary units), the time from appearance of stereotype behaviour till the complete cessation of it was expressed as the duration of stereotypy (min).

Statistical analysis

Through the text the data are presented in mean values, with standard errors of the mean ($x \pm$ S.E.M.). Statistical significance of differences between mean values was evaluated by Mann-Whitney U test for the intensity of the stereotypy and by two-tailed Student's test for its duration. A p value of less than 0.05 was considered to be significant.

Drugs

(−) Deprenyl HCl (Chinoin, Hungary) was dissolved in physiological saline, J-508 (Chinoin, Hungary), clorgyline (May and Baker, England)

and phenylethylamine (Chinoin, Hungary) were dissolved in distilled water.

Results

Effect of graded doses of MAOIs

Single administration of small doses of (−) deprenyl one hour prior to PEA potentiated the PEA-induced stereotypy in a dose related manner (Fig. 1). Pretreatment with 0.1 mg/kg (−) deprenyl increased significantly the intensity of PEA stereotypy but the change in the duration was not significant, whereas 0.25 mg/kg (−) deprenyl significantly enhanced both

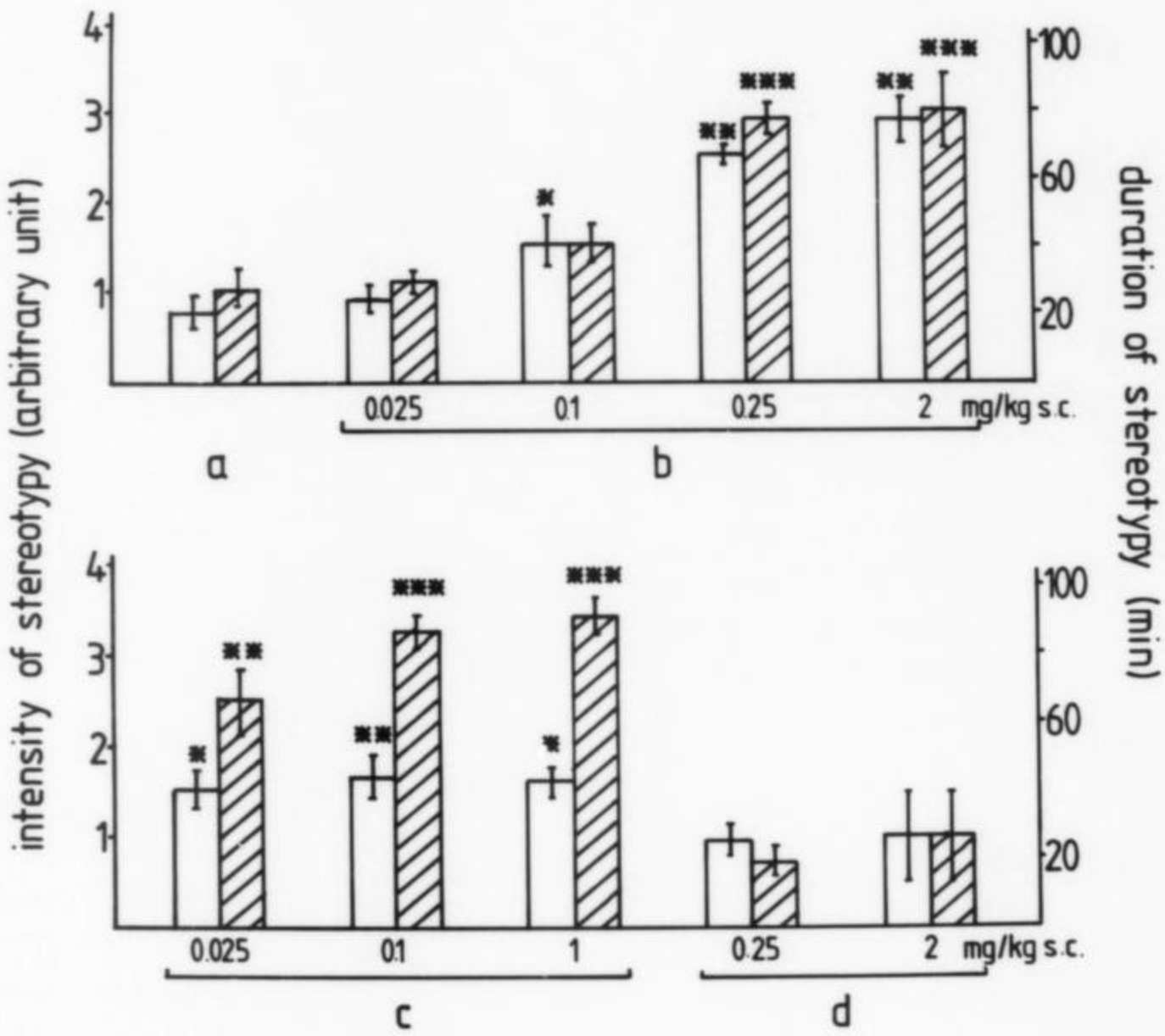

FIG. 1

The effect of single MAOIs administration on PEA-induced stereotype behavour.

a – physiological (−) saline + PEA, b – (−) deprenyl + PEA, c – J-508 + PEA, d – clorgyline + PEA. Pretreatment one hour prior to 40 mg/kg s.c. PEA. Empty bars – the intensity of stereotypy; stripped bars – the duration of stereotypy. Each bar represents the mean ± S.E.M. of 8–10 animals. Significancy is calculated to the group treated with physiological saline + PEA.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

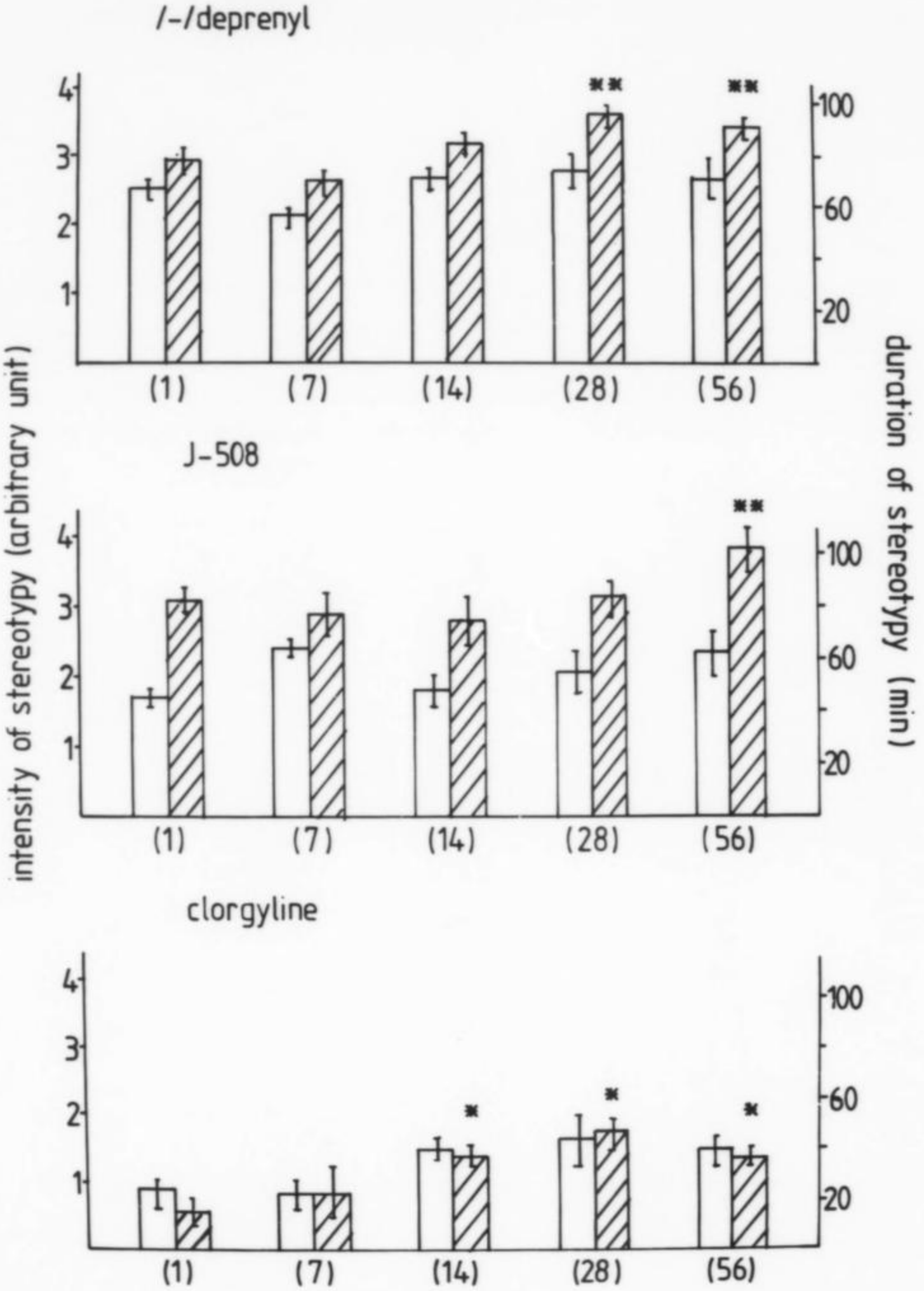

FIG. 2

The effect of repeated administration of MAOIs on PEA-induced stereotypy.

The MAOIs were injected once daily s.c. (/-/deprenyl and clorgyline 0.25 mg/kg, J-508 0.1 mg/kg). PEA (40 mg/kg s.c.) was administered 24 hr after the last MAOI injection. Meaning of bars see under Fig. 1. Numbers in brackets indicate the daily injections before testing. Significancy is calculated to the effect of single dose.

*$p < 0.05$, **$p < 0.01$.

the intensity (2.5 ± 0.17 versus 0.8 ± 0.13 in physiological saline treated animals) and the duration (78.0 ± 3.59 versus 27.0 ± 5.59) of stereotype behaviour. The increase of the dose of (−) deprenyl was no more effective, the enhancement in the PEA-induced stereotypy after 2 mg/kg of (−) deprenyl was the same as with 0.25 mg/kg.

J-508 has similar potentiating effect on the PEA-induced stereotypy, however, its effect on the intensity (comparing either the small or the higher doses) was significantly smaller than that of (−) deprenyl ($p < 0.05$). The duration of the stereotypy increased as much as in the case of (−) deprenyl. With 0.1 mg/kg J-508 the upper limit of the potentiation of PEA effect was reached. Clorgyline did not cause any change in PEA-induced stereotypy either in its selective (0.25 mg/kg), or higher dose (2 mg/kg).

The effect of repeated administration of MAOIs

Repeated administration of selective doses of MAO-B inhibitors daily even for 8 weeks did not really change more the intensity of PEA-induced stereotypy than single doses (Fig. 2). There was a small, mathematically significant increase in the duration of stereotypy on the 4th and 8th week of the treatment in the case of (−) deprenyl (96.0 ± 1.67 and 88.9 ± 3.09 versus 79.9 ± 3.75) and on the 8th week in the case of J-508 (104.0 ± 2.67 versus 87.8 ± 3.35). Repeated administration of the MAO-A inhibitor, clorgyline, caused a small, mathematically not significant increase in the intensity of the PEA-induced stereotypy on the 2nd week (1.5 ± 0.17 versus 0.9 ± 0.28), the duration of which was significantly increased (36.67 ± 2.36 versus 15.0 ± 4.77). However, this weak PEA potentiating effect did not increase during further treatment.

The offset of the action of long-lasting MAO-B inhibitor pretreatment

Stopping the treatment with the MAO-B inhibitors at the end of the 8th week the cessation-time of the PEA potentiating effect was investigated (Fig. 3). The enhancement of the intensity and duration of the PEA-induced stereotypy decreased slowly. Even after a two-week washing period both the intensity and the duration of the PEA effect was significantly higher than in untreated rats. In (−) deprenyl treated animals (but not in J-508 treated ones) the intensity of PEA-induced stereotypy remained significantly higher even after 4 weeks of withdrawal (1.67 ± 0.17 versus 0.8 ± 0.13). On the 6th week there was no significant difference in the effect of PEA compared to the untreated group.

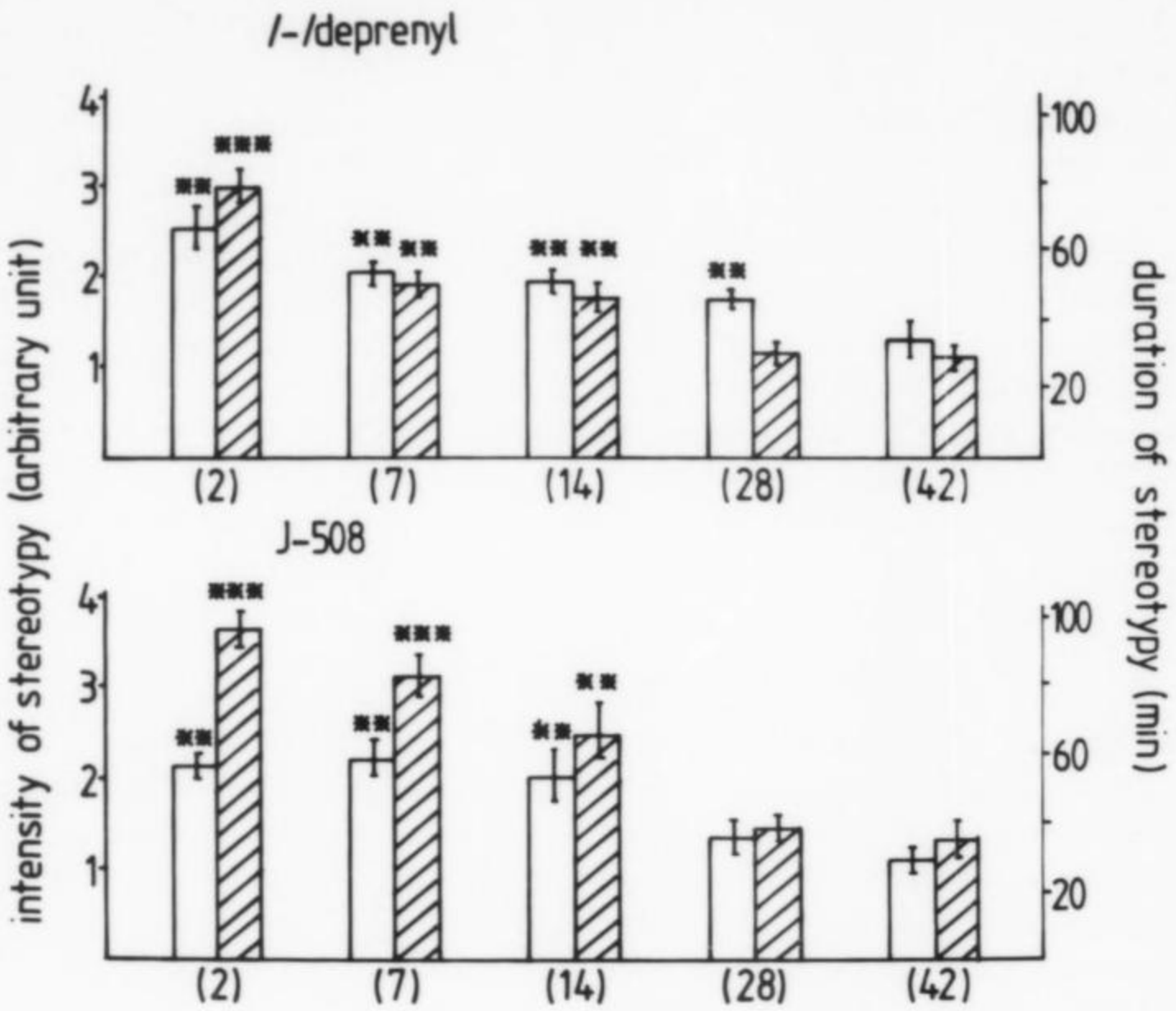

FIG. 3

The offset of the action of chronic pretreatment with MAO-B inhibitors on the PEA-induced stereotypy. Pretreatment—0.25 mg/kg (−) deprenyl or 0.1 mg/kg J-508 s.c. once/daily for 8 weeks. Meaning of bars see under Fig. 1. Numbers in brackets indicate the days passed from the last injection to the testing day. The dose of PEA was 40 mg/kg s.c. Significancy is calculated to the effect of PEA after physiological saline (see Fig. 1/a).

*$p < 0.01$, ***$p < 0.001$.

Discussion

PEA, a specific substrate for MAO-B (Yang and Neff, 1973; Neff *et al.*, 1974) is rapidly oxidized by this enzyme (Durden and Philips, 1980). Knoll (1976) demonstrated that a single i.v. dose of 0.25 mg/kg (−) deprenyl, due to the inhibition of MAO-B activity in the liver, enormously potentiates the effect of PEA on the cat nictitating membrane, whereas the inhibition of MAO-A by clorgyline did not influence the effect of PEA.

The same fits for the brain. According to Mosnaim and Wolf (1978) 10 min after i.c.v. administration of labelled PEA a measurable amount of metabolites is formed and accumulated in rabbit's brain. The potentiation of the effect of PEA on the central nervous system by (−) deprenyl was

demonstrated in different species (Braestrup *et al.*, 1975; Gianutsos *et al.*, 1983). Braestrup used (−) deprenyl in a dose of 2 mg/kg to potentiate the stereotype effect of PEA and found that clorgyline was ineffective. Ortmann *et al.* (1984) used 3 mg/kg (−) deprenyl to induce the PEA syndrome. The data presented in this paper clearly show that the use of (−) deprenyl over the selective, 0.25 mg/kg dose, which dose inhibits the PEA deamination by about 70 % (Neff *et al.*, 1974), has no further effect on the PEA-induced stereotypy. The use of high doses of (−) deprenyl are unnecessary and groundless. (Ortmann found the smaller dose of (−) deprenyl to be ineffective on inducing PEA syndrome, the cause of it may be the different method used for examination of stereotypy.)

It is well known from previous studies on (−) deprenyl that there are a number of pharmacological effects which can be detected only during repeated administration of the drug (for review see Knoll, 1983). For example (−) deprenyl increases the turnover rate of dopamine in the striatum in rats, pretreated with a daily dose of 0.25 mg/kg (Zsilla and Knoll, 1982), whereas this effect can not be detected after a single dose of the drug. Another example for a difference in the pharmacological effects between a single and repeated doses of (−) deprenyl is the change in the imipramine binding sites in rat brain cortex and hyppocampus. Whereas a 3 weeks pretreatment with 0.25 mg/kg (−) deprenyl significantly increased the imipramine binding capacity of the brain, no change was detected after a single dose of the drug. All these changes are due to more complex pharmacological effect of (−) deprenyl and can not be reproduced by compounds having only MAO inhibitory potency. Pargyline, which is as potent as (−) deprenyl in inhibiting MAO, was found to be ineffective in changing imipramine binding in the rat brain even when given in 100 times higher doses than (−) deprenyl (Zsilla *et al.*, 1983).

With this in mind we compared the effect of a single dose to that of 8 weeks daily pretreatment with the same dose of (−) deprenyl and found that with regard to its effect on the PEA-induced stereotypy there was no difference between these two groups of animals, indicating that the facilitation of PEA-induced stereotypy by (−) deprenyl is completely due to the inhibition of B-type MAO in the brain.

The other MAO-B inhibitor we investigated, J-508, is an about 20 times more potent inhibitor of MAO than (−) deprenyl. In the applied dose (0.1 mg/kg) given acutely it inhibits completely the deamination of PEA in the rat brain, however, there is an about 30 % reduction in the 5-HT metabolism as well (Knoll *et al.*, 1978). Following a one week repeated administration of this dose, 5-HT deamination is nearly completely blocked (Ekstedt *et al.*, 1979). As J-508 influences not only the PEA metabolism, but also the metabolism of other amines deaminated by MAO-A, the increased content of these amines (for example 5-HT) may modify the run of PEA-induced stereotypy. This may explain the small differences in the

PEA potentiating effect of the two inhibitors, i.e. the PEA-induced stereotypy potentiated by J-508 proved to be smaller as achieved by (−) deprenyl following either single or repeated administration.

The inhibition of PEA metabolism elicited by repeated administration of MAO-B inhibitors proved to be long-lasting. These drugs are published to be irreversible "enzyme-killing" inhibitors (Knoll, 1981), as on the basis of our results the complete restauration of the PEA metabolism needs about 4–6 weeks.

Investigating the effect of (−) deprenyl on stereotype behaviour the possible role of its metabolites, amphetamine and metamphetamine (Reynolds *et al.*, 1978) can not be left unregarded. In our experiments (−) deprenyl failed to evoke any sign of stereotype behaviour either in high dose (20 mg/kg) or following repeated administration. According to Braestrup (1975) (−) deprenyl induced a weak amphetamine-like behavioural stimulation only in a dose of 100 mg/kg. It is evident that the metabolites of this small dose of (−) deprenyl have no pharmacological effects at all. This is in good agreement with previous detailed studies (Knoll, 1980, 1983).

The data presented in our study speak in favour of the extension of clinical studies with (−) deprenyl in combination with phenylalanine, the precursor of PEA in depressed patients. (−) Deprenyl might play the role of a useful experimental tool in the development of a new biochemical classification of depressive disorders. The first clinical attempts in this direction are promising (Mann *et al.*, 1982).

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Received March 11, 1985.